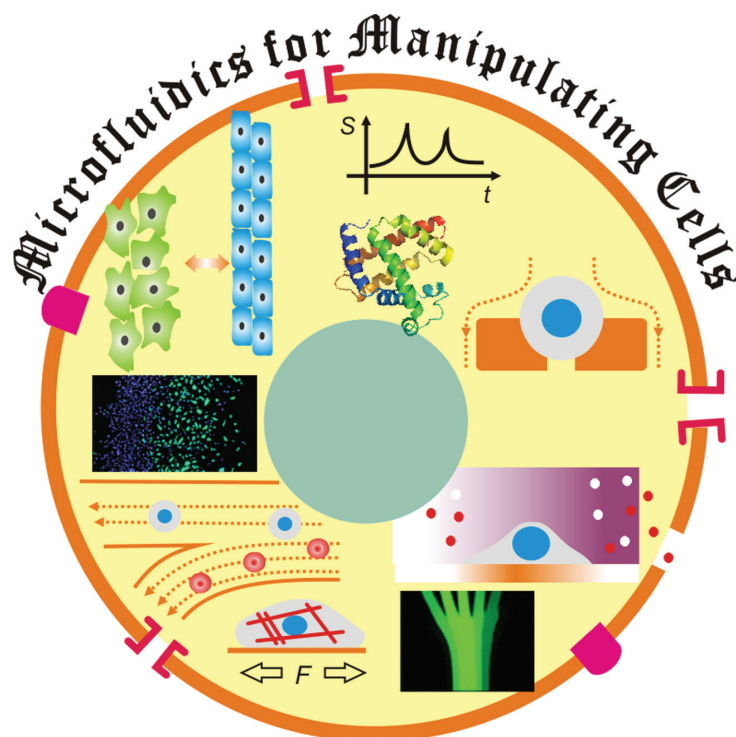


Microfluidics for Manipulating Cells

Xuan Mu, Wenfu Zheng, Jiashu Sun, Wei Zhang, and Xingyu Jiang*



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Microfluidics, a toolbox comprising methods for precise manipulation of fluids at small length scales (micrometers to millimeters), has become useful for manipulating cells. Its uses range from dynamic management of cellular interactions to high-throughput screening of cells, and to precise analysis of chemical contents in single cells. Microfluidics demonstrates a completely new perspective and an excellent practical way to manipulate cells for solving various needs in biology and medicine. This review introduces and comments on recent achievements and challenges of using microfluidics to manipulate and analyze cells. It is believed that microfluidics will assume an even greater role in the mechanistic understanding of cell biology and, eventually, in clinical applications.

1. Introduction

Microfluidics typically refers to the precise control of minute amounts of fluids using micro-scale structures.^[1] Its concept dates back to 1970s^[2] and starts to become popular in 1990s^[3,4] as a form of miniaturized platforms for bio-analysis. Microfluidics, mainly relying on its small scale, could create numerous new opportunities to manipulate and analyze cells.^[5] The inherent advantages of scale effect on microfluidics include: 1) Suitable scale. The size of channels and structures in microfluidic chips usually ranges from several to hundreds of micrometers, a scale close to that of cells allowing convenient manipulations of cells. 2) Unique fluidic phenomena. Various fluidic phenomena under small scale like laminar flow and microscale droplet could implement many manipulations that are hard or impassible for conventional, macroscopic methods to achieve. Besides, hydrodynamics might provide a label-free means to screen cells. 3) Integration of multiple functions. It is straightforward to fabricate multiple microstructures with high density. Although it does not always lead to multi-functionality, the capability of integration of multi-structures shows huge potential to integrate multiple functions in parallel or cascade. Therefore, it is possible to transform complex procedures, for example single-cell analysis, into a simple run on microfluidic chips. 4) Large surface area. Microfluidic chips often possess much larger surface-to-volume ratio than conventional apparatus, facilitating surface-dependent applications like capturing cells by affinity. All these advantages make microfluidics a compelling workhorse to carry out unprecedented scientific investigations and reveal profound biological insights.^[6–8]

Microfluidics has been applied in many aspects of cell biology, and reviewed in a number of excellent papers with focused emphases.^[9–13] However, this field has developed quickly during the last years with many new achievements, which deserves a newer review with a specific perspective.

Due to constrained space, we will mainly focus on four subfields among the diverse achievements in microfluidics: Cell Patterning, Microenvironment Patterning, Cell Screening and Single-Cell Analysis. (Figure 1). Section 2 shows the spatial and temporal manipulation of cells and extracellular matrixes; Section 3 describes the efforts in microfluidics to screen cells with enhanced sensitivity and throughput; Section 4 demonstrates the integrated steps of single cells analysis on microfluidic chips. We also feel it is necessary to introduce how to maintain cell viable in microfluidic channel in a long period in Section 5. We expect to provide typical examples, recent feats, and challenges of microfluidics in manipulating cells to new scientists in this field as well as others in relevant fields.

2. Cell and Microenvironment Patterning

We use “cell patterning” to refer to the confinement of individual or a group of cells to rational, predesigned geometrical shapes, while we use “microenvironment patterning” to refer to the spatio-temporal control of various environmental cues surrounding the cells. The cell and microenvironment

patterning allows constructing *in vitro* models to mimic and investigate *in vivo* cellular processes and cell-cell interactions, such as cellular apoptosis,^[14] differentiation,^[15] and signaling,^[16] for investigating tissue regeneration,^[17,18] cancer invasion,^[19–23] neuronal development,^[24,25] and drug screening.^[26–30]

To pattern cells and microenvironment, there is an iconic method in microfluidics, laminar flow patterning (LFP),^[31–33] which is a representative fluidic phenomenon in microfluidic channels governed by viscous force dominating over inertial force. Laminar flow of multiple streams could pattern cells,^[34–36] various soluble cues,^[37–45] as well as temperature.^[46] Although laminar flow shows strong capabilities of patterning,^[47] the distance of patterning is limited, because transverse diffusion would obscure the interface between each stream after certain distance (typically < 2 cm).^[48] Besides the limited distance, there are also challenges in patterning that should be dealt with, such as diverse shapes, dynamic control of cell adhesion, constructing alterable microenvironment, as well as generating patterns in 3D. These challenges are crucial to manipulating cells in demand and for mimicking physiological process. We discuss current achievements in microfluidics to tackle these challenges in Sections 2.1 and 2.2.

2.1. Cell Patterning

2.1.1. Microchannel Patterning

Microfluidic channels could pattern cells on a surface in shapes of microchannels with micrometer precision.^[49,50] After adhesion of cells (different cells in different microchannels), peeling off microfluidic channels could lead in predesigned co-culturing, this is especially useful for studying cell-cell interactions. Compared with Transwell, a commonly used device to study cell-cell interactions, microfluidics enables patterning on a surface and thus real-time observations. We employed this method to investigate the pathogenesis of endometriosis that is closely involved with interactions between endometrial stromal cells (ESCs) and human peritoneal mesothelial cells (HPMCs).^[59]

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Patterning inside microchannels is also an important topic. Specific structures,^[51,52] DNA hybridization,^[53] surface chemistry like electrochemical desorption of thiol,^[54–56] photoliable polymer^[57] and photo-tunable azobenzenes,^[58] could create versatile patterns within microchannels, serving as a set of powerful tools. Kitamori *et al.* employed a photoliable polymer to control the size of cell pattern, which could maintain for 5 weeks.^[57] We placed a perforated polydimethylsiloxane (PDMS) membrane between the patterned surface and microfluidic channels to pattern multiple types of cells (**Figure 2A**).^[60] This approach allows patterning of multiple types of cell for the mimicry of wound healing. An extension of this work is to selectively damage cells with low osmotic pressure, and to study how the epithelial cell are covered from lateral channels.^[61] The other effective way of microchannel patterning is to construct particular regions by microwells within microchannels, and capture and culture multiple cells, such as hepatocytes and embryonic stem cells (**Figure 2B**).^[62]

The dynamic control of the location and motility of cells on a surface could offer more versatility and become increasingly important for tissue engineering and pharmacological screens.^[47,63] We combined microchannel patterning and surface chemistry to pattern cells to mimic three kinds of cell-cell interactions in one platform:^[64,65] 1) both kinds of cells are kept confined; 2) one cell can migrate while another is restricted and immobile; 3) two types of cells can move freely. The stimulation of multiple *in vivo* scenarios of cell-cell interactions could enable modeling of complex physiological processes.

2.1.2. Patterning in 3D

Patterning cells on 2D surfaces is significant for fundamental cell biology research, but is limited in mimicking key physiological structures in 3D. We developed a stress-induced rolling membrane (SIRM) to transfer patterns of live cells on 2D surfaces into blood vessel-like 3D tubular structures (**Figure 2C**).^[66] The PDMS scaffold using SIRM contributes to the stability of the biomimetic blood vessel. Because of the high structural similarity to many tubular tissues and the stability of the tubular shape, this artificial tube has great potential to mimic both normal and pathological blood vessels.

Patterning cells in 3D hydrogels is of great significance to model *in vivo* physiologic conditions. Assembling cells into hydrogel^[67–72] could provide deep insights into cancer invasion and angiogenesis. Fabricating microfluidic chips with hydrogel is impressive for mimicking the vasculature.^[73,74] However, the microfluidic biomimetic vasculature is hard to realize controlled structure and cytocompatibility simultaneously, which prevents from mimicking vasculature-involved physiological functions like mass transfer between vessel and tubule.

2.2. Microenvironment Patterning

Microfluidics could not only be used for manipulating cells, but also for constructing and regulating their surrounding



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microenvironment with the refined controlling of both soluble and mechanical cues.

2.2.1. Soluble Cues

The delivery of chemical stimuli to cells is extremely helpful to obtain profound insights into cell-cell and endocellular processes. Microfluidics could directly deliver soluble cues to a specific subgroup of cells or part of single cells. Ismagilov *et al.* employed LFP to treat specific regions (tens of micron) on a plant root (*Arabidopsis thaliana*) with a plant hormone auxin derivative (**Figure 2D**).^[42] This platform could reveal the mechanism of plant hormone-mediated root development and apply to many other aspects of root biology. Wu *et al.* delivered plasmids to transfect different fluorescence proteins to different locations within single Cos-7 cells.^[45] This work enables studies of cell behaviors with two exogenous transfections in the same culture.

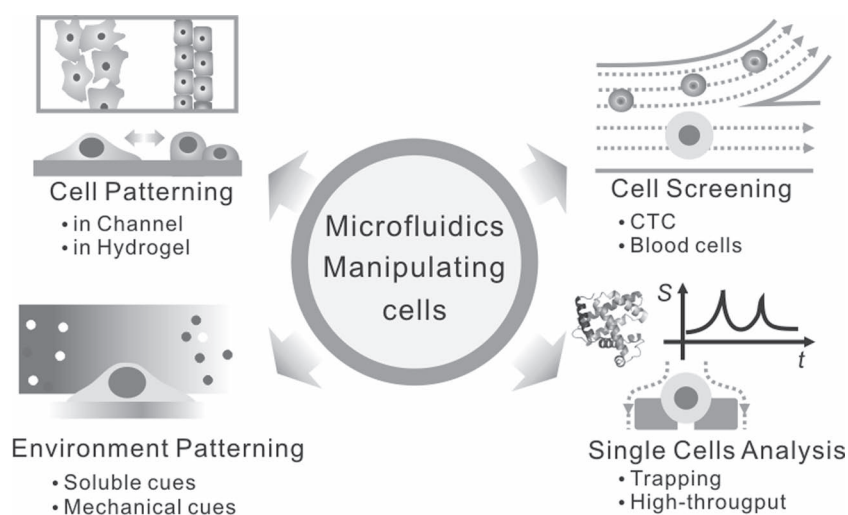


Figure 1. Microfluidics could contribute to manipulating cells in the following aspects: cell and microenvironment patterning, cell screening and single cell analysis. Either aspect greatly benefits from the intrinsic merits of microfluidics.

In addition to precise delivery to certain locations, microfluidics could also form gradients to mimic the natural physiological distribution of signal molecules across tissues.^[75–77] We developed a microfluidic network for serial dilution^[78] and a folded microfluidic network integrated with several chaotic advective mixers to generate molecular gradient.^[79] Next, we constructed a modular microfluidic chip for generating arbitrary gradients.^[80] The modular components of the microfluidic chip involve distributors, combiners, resistors and collectors, which connect with tubes into a network. This chip enabled real-time manipulation of gradient profiles, and

a simple implementation for non-specialists. Wu and Zare *et al.* employed agarose hydrogel to build various complex and static gradients of diffusible molecules in static solutions along microfluidic channels.^[81] Another example of microfluidics in patterning soluble cues is to develop a periodically changed culturing environment (Figure 2E),^[82–84] which is extremely useful to develop synthetic gene oscillator and its entrainment.

2.2.2. Mechanical Cues

Cells can impose forces on extracellular microenvironments, and mutually sense surrounding mechanical cues.^[85] The mechanical cues can lead to the initiation of signal pathways and the alteration of gene expressions and functions of cells.^[86] The typical mechanical cues include stretching, shear stress, and rigidity of substrate. Microfluidics could reconstruct these cues to cells in a precise and real-time manner.^[87–89]

Stretching cells is usually realized by pulling the deformable substrate where cells are attached to. We constructed a microfluidic device to observe cells being stretched in real-time under a 63× oil objective.^[90] Such high magnification allows the resolution of single actin fibrils, but previous methods of stretching did not allow the real-time observation under such magnification because they required longer observing distance than that of microscopic objectives at this magnification. The device was employed for studying

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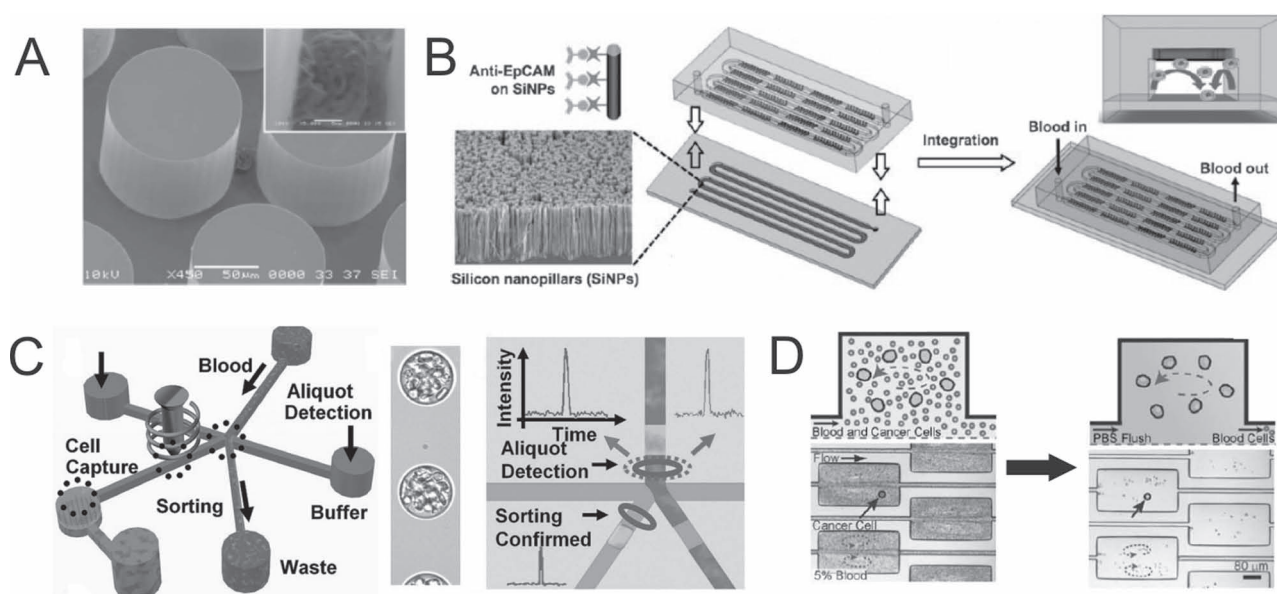


Figure 2. Isolation of CTCs on microfluidic chips. A) One CTC (enlarged in inset) adheres to the anti-body coated post in a microfluidic channel. Reproduced with permission.^[128] Copyright 2008, Nature Publishing Group; B) Schematic illustration of the configuration and operation of microfluidic device to trap CTCs with chaotic mixer to enhance efficiency and sensitivity. Reproduced with permission.^[139] C) Schematics and operation of ensemble-decision aliquot ranking. Reproduced with permission.^[100] D) Capture CTCs by vertices in microfluidic channels. Reproduced with permission.^[117] Copyright 2012, The Royal Society of Chemistry.

molecular dynamics in the process of mechanotransduction. Huh *et al.* devised a microfluidic chip to mimic the distention of alveoli of the human lung during inhalation. Their results show the stretching could accentuate the toxic and inflammatory responses of the cells to silica nanoparticles.^[91]

Another type of mechanical stimulation to cells is shear stress that influences cell adhesion, cytoskeletal alignment, as well as gene expressions.^[92] Microfluidic devices could generate a range of shear stresses over two orders of magnitude to cells,^[93] and study the response of mesenchymal stem cells (MSCs).^[94] Due to the rather complex mechanical environment *in vivo*,^[95] an ideal *in vitro* mechanical stimulation model must mimic multiple forces acting on the cells. We reported a microfluidic device to combine both shear stress and stretch to mimic hemodynamic microenvironment of blood vessels.^[96]

The rigidity of cell adherent substrate has important implications on development and differentiation of cells.^[97] Chen *et al.* observed cells would migrate to stiffer regions of substrate.^[98] LeDuc *et al.* employed microfluidic channel to pattern alternative rigidities on substrate. Their results show that localized rigidity would restrict the outgrowth and orientation of neurite in differentiating neuroblasts.^[99]

3. Cell Screening

The screening of cells is of particular interest, extremely important in cell biology and often the first step in routine clinical assays. It is also a challenging task. The most widely used apparatus in cell screening is flow cytometry. But this technique has some drawbacks such as high cost, mechanical complexity, difficulty for maintaining, large sample volume, and insensitivity to some rare cells. The microfluidic chips have great potential to address these challenges and have become useful for both clinical and fundamental applications.

The principles of cell screening employed in microfluidics are diverse. Some works applied fluorescence-activated sorting to microscale for miniaturizing conventional flow cytometry on microfluidic chips.^[100–102] Other employed principles include dielectrophoresis,^[103,104] magnetic force,^[105–108] filtration,^[109,110] and droplets.^[111–114] Droplet in microfluidic chips is a high throughput method and could vastly contribute to cell screening.

Besides, microfluidics has two inherent advantages to screen cells: large surface-to-volume ratio and hydrodynamic effect. The large surface-to-volume ratio of microfluidic chips enable the highly efficient capture of cells by using antibody,^[127–129] DNA-aptamer^[130–132] dendrimer^[133] and imprinted structure.^[134–136] Nanostructures^[137,138] and appropriate turbulence^[139–141] could assist in improving the efficiency of capture. Hydrodynamic effects could rapidly sort cells by their different physical properties, such as size and density, and consequent fluidic behaviors of cells in fluid flow.^[115–126] This method provides the opportunity to isolate cells from others in continuous flow and in a label-free manner.

To compare the current methods for isolating cells, we use a table to document them in four aspects: enrichment, throughput, efficiency and purity (**Table 1**). According to this

table, affinity-based capture affords the highest capture efficiency (of 100%), while the hydrodynamic effect provides the highest enrichment (of 3×10^6) and purity (of 100%).

In the following sections (3.1 ~ 3.3), we would mainly introduce the current application-specific development of cell screening in terms of targeting cells: cancer cells and blood cells.

3.1. Cancer Cells

Circulating tumor cells (CTCs) exist in the peripheral blood of cancer patients, but are extremely rare, accounting for approximately $1-10$ of all blood cells.^[142,143] The detection of CTCs could indicate metastatic diseases of cancers, providing information regarding cancer diagnosis and prognosis. However, conventional methods for isolating rare CTCs involve laborious procedures and expensive instruments, such as CellSearch, with lack of specificity and efficiency because of the low levels of CTCs and the variable expression of marker molecules on CTC surface. Microfluidic chips have become an increasingly important platform to separate CTCs from normal cells.^[132,144–147]

Toner *et al.* developed microfluidic chips with dense arrays of microposts coated with anti-epithelial cell adhesion molecule (EpCAM), an antibody to CTCs (**Figure 3A**).^[128] They identified and isolated viable rare CTCs from peripheral whole blood samples of patients of metastatic lung, prostate, pancreatic, breast and colon cancer in 115 of 116 (99%) clinical samples and with 50% purity. Wang and Tseng *et al.* demonstrated that silicon nanopillars coated with EpCAM could improve the trapping of CTCs in artificial blood.^[138] They integrated it with a microfluidic chaotic mixer to further enhance the efficiency and sensitivity, and thus the performance of trapping (**Figure 3B**).^[139,141] The mixing could induce vertical flow that significantly improves the frequency of cell-substrate interaction, allowing the capture of more than 95% of CTCs in real patient samples. Chiu *et al.* developed an approach of ensemble-decision aliquot ranking (eDAR) to isolate CTCs from breast cancer patients^[100] in a side-by-side comparison with a commercialized product CellSearch (**Figure 3C**).^[148] The spontaneously generated vortices on microfluidic chips could also isolate CTCs (**Figure 3D**), featuring automated preparation of cell sample, as a potential alternative to standard bench-top centrifuge.

3.2. Blood Cells

Blood cell is a combination of multiplex subgroups of cells, including red blood cells (RBCs), platelets and white blood cells (WBCs). For only WBCs, it could be further divided into 5 classes: neutrophils, lymphocytes, monocytes, eosinophils and basophils. The wide diversity of cell types is a challenge that restricts and interferes the extracting of information from one specific type of cells.^[149] Therefore, the isolated cells from blood should be as pure as possible.

Microfluidics has been employed in separation of blood plasma,^[150,151] erythrocyte,^[122,152,153] platelets,^[121,154] and leukocytes.^[155] All these works achieve adequate efficiency of isolation that could satisfy downstream analysis like PCR.

Table 1. Screening of cells on microfluidic chips.

Types	Principles ^{a)}	Fold of Enrichment	Throughput ^{b)}	Capture Efficiency	Purity ^{c)}	Ref.
<i>Bombyx mori</i>	D, E	–	125 ± 40 cells/s	–	–	[111]
CTC	A	135	300 nL/s	83 ± 9%	96%	[130]
CTC	A	–	1 ml/40 min	96 ± 4%	–	[129]
CTC	D, F	–	25–80 ul/min	100–85%	10–50%	[100]
CTC	A, N	–	1 ml/h	95%	–	[139]
CTC	A, M	–	10 mL/h	90–86%	–	[108]
CTC	A	–	600 nl/s	95%	~81%	[132]
CTC	H	3 × 10 ⁶	5 mL/min	20%	~40%	[117]
CTC	H	3.25 × 10 ⁵	2.8 × 10 ⁴ cells/s	~ 80%	–	[122]
<i>E. coli</i>	H	300	5.7 × 10 ⁴ cells/s	62%	99.87%	[120]
<i>E. coli</i>	F	83–90	44 cells/s	50%	~100%	[157]
<i>E. coli</i>	E	~1000	~4 × 10 ³ cells/s	–	4–34%	[158]
<i>E. coli</i>	M	500	~2.8 × 10 ⁵ cells/s	–	90%	[106]
<i>E. coli</i>	E, M	900	~7 × 10 ³ cells/s	–	95%	[104]
<i>E. coli</i>	D, E	–	300–2000 droplets/s	99%	99%	[112]
Leukocyte	H	34	136 pl/s	–	–	[155]
Lymphocytes (B cells)	A	600	100 µL/min	–	99%	[127]
Lymphocytes (Jurkat T cell)	H	1 × 10 ⁴	160 cells/s	70–80%	94 ± 3%	[123]
Nucleated cells	H	45.75 ± 2.5	5 nl/s	94 ± 4.5%	90–99%	[119]
Neurons	H	–	~1.7 × 10 ⁴ cells/s	90%	60–70%	[161]
Platelet	H	100	1 ml/min	–	90–100%	[121]
RBC, WBC	H	–	1 × 10 ⁶ cells/s	94% (RBC) 86% (WBC)	97% (RBC) 88% (WBC)	[156]
Yeast	D, F	–	2000 droplets/s	–	–	[113]
Yeast	D, H	–	1 × 10 ⁴ droplets/s	–	97%	[114]

^{a)}A=affinity; D=droplets; E=dielectrophoresis; F=Fluorescence; H=hydrodynamics; M=Magnetism; N=Nanostructures; ^{b)}the unit of throughput cannot be unified due to the different samples in literatures; ^{c)}some values are calculated or estimated based on the data in literatures by the authors.

One excellent work of microfluidics in blood cells screening is to separate and count RBCs and WBCs in a high throughput manner.^[156] The microfluidic chip has 256 straight parallel channels that make the sampling rate as extremely high as one million cells per second.

Another challenge in isolating blood cells is to maintain the immune-phenotype of leukocytes, since they can rapidly respond to the alteration of the environment. The optimization of the design of microstructures^[109] and soft fluid force^[120] may minimize this defect.

Apart from cancer and blood cells, the versatile applicability of microfluidics is also reflected in the screening of *E. coli*,^[104,106,120,157,158] embryonic bodies (EBs),^[159] neurons,^[160,161] fungi^[162] and embryonic kidney cells (HEK293).^[163]

4. Single Cell Analysis

The analysis of biochemical contents, such as protein,^[164] DNA and RNA,^[165] in single cells, is critical for the understanding of cellular processes.^[166] Unlike conventional

analysis for a batch of cells, single-cell analysis could avoid the loss of information associated with ensemble averaging, and thus reflect important variations in a cellular population. Microfluidics has the ability to streamline the complex analysis procedures with rapid, accurate, and cost-effective on-chip operation and^[167] multiplexed measurements.^[168,169] The sections (4.1 and 4.2) will review challenges and achievements of microfluidic single-cell analysis in two aspects: high-throughput and trapping.

4.1. High-Throughput

High throughput is important to provide population statistics to exclude rare events, but it is a challenge to maintain single-cell precision in a high-throughput manner, especially for batch apparatuses. The current high-throughput microfluidic approaches include multiple parallel microchannels,^[170] microfluidic image cytometry (MIC)^[171–173] and droplets.^[174–176] Xie *et al.* achieved a screening rate of ~ 4000

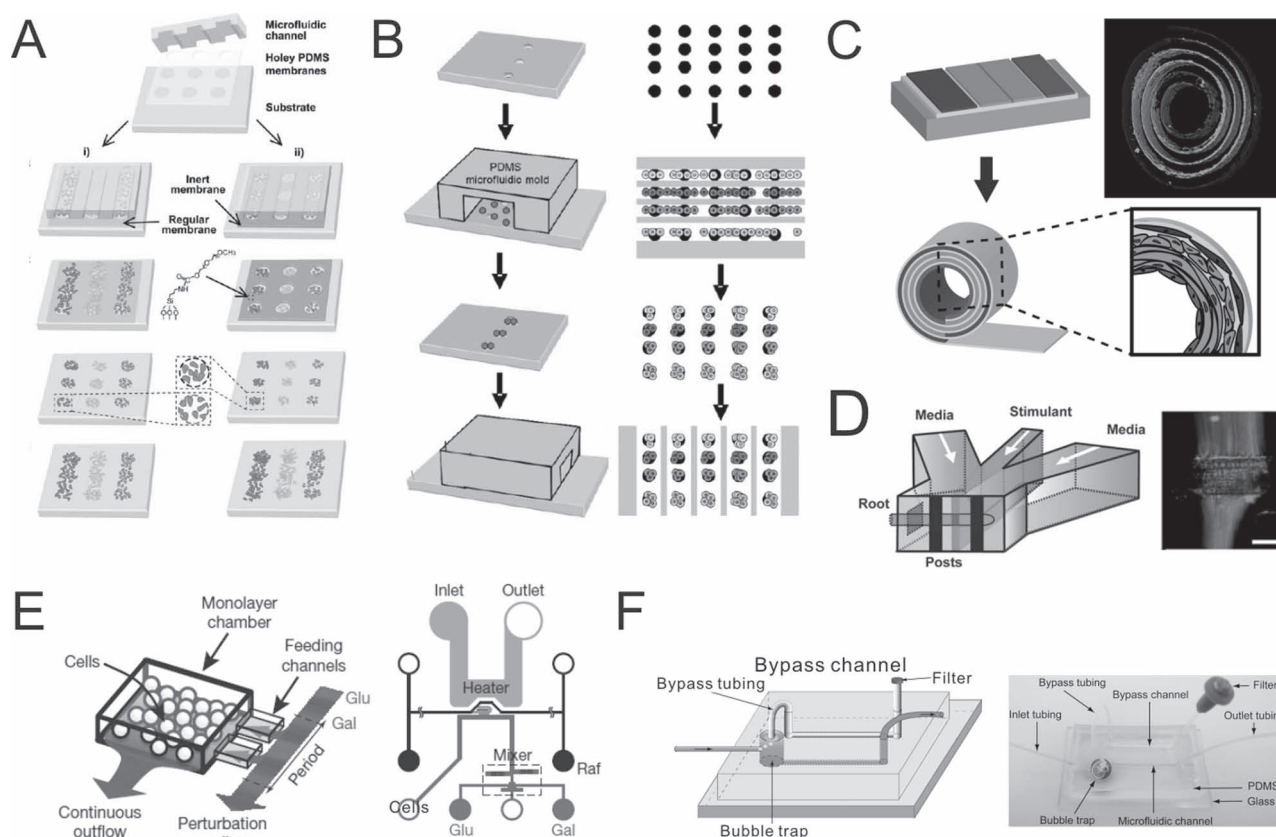


Figure 3. A) Patterning multiple cells by microfluidic channels and perforated PDMS membrane for studying wound healing. Reproduced with permission;^[60] B) Docking cells with microfluidic channel for multiphenotype cell arrays. Reproduced with permission.^[62] Copyright 2005, The Royal Society of Chemistry; C) Stress-induced rolling membrane (SIRM) for transferring 2D cell patterning to 3D tubule-like structure, having intima composed of endothelial cells, media of smooth muscle cells, as well as adventitia of fibroblast. Reproduced with permission;^[66] D) Schematics of microfluidic platform for regional chemical stimulate to a live *Arabidopsis* root. The right image (scale bar 75 μ m) shows the streamlines around the root segment.^[42] Copyright 2010, The Royal Society of Chemistry; E) The design of a microfluidic chip to create sinusoidal perturbations in the glucose (Glu) concentration while maintaining constant background levels of galactose (Gal). Reproduced with permission.^[82] Copyright 2008, Nature Publishing Group; F) Schematics and photograph of integrated bubble-trap microfluidic device for long-term cell culture. Reproduced with permission.^[218] Copyright 2010, The Royal Society of Chemistry.

cells in 25s (**Figure 4A**).^[170] Mathies *et al.* generated 3.4×10^6 droplets per hour and detect one pathogenic *E. coli* O157 cell from a background of 10^5 normal K12 cells.^[174]

Another challenge for single cells analysis is to trap single-cell of interest from a swarm of cells, and represents a prerequisite to single-cells analysis. The next section (4.2) would introduce four typical means for trapping single cells on microfluidics chips (**Table 2**).

4.2. Cell trapping

4.2.1. Microarray

One method to trap single cells in microfluidics is the microarray composed of multiple replicated microstructures. The microarrays usually have U-shaped structures with optimized parameters for trapping.^[177–182] Some studies demonstrated the efficiency of trapping could be 95%^[183,184] (Figure 4B) and 97%.^[185] These platforms also enable single cell co-culture^[186,187] and on-site analysis of the concentration, kinetics and inhibition of enzymes in single cells.^[188,189]

4.2.2. Integrated Valve

The second way to trap single cells is to employ pneumatic valves integrated in microfluidic chips.^[190–193] One advantage of integration is that the contents in cells are separated before detection.^[194,195] The separated bands of chemical contents in single cells could be compartmentalized in droplets to avoid dilution and the loss of separated components.^[196] Zare and colleagues designed and fabricated a cell dock to gently trap single cells for analyzing cellular viability and Ca^{2+} flux.^[190] They developed a novel valve having three states, open, close and half-open, to replace a number of conventional two-state valves and minimize the final reaction volume to a mere 70 pL.^[191] They further separated and analyzed low-copy-number proteins in individual cells.^[197] Wang *et al.* employed pneumatic valves to reversibly trap and release a small group of cells with high potential to apply to single cells.^[198] The trapping of cells could also circumvent efflux problem for analyzing the stochastic expression of enzymes in single molecule level in individual cells (Figure 4C).^[199]

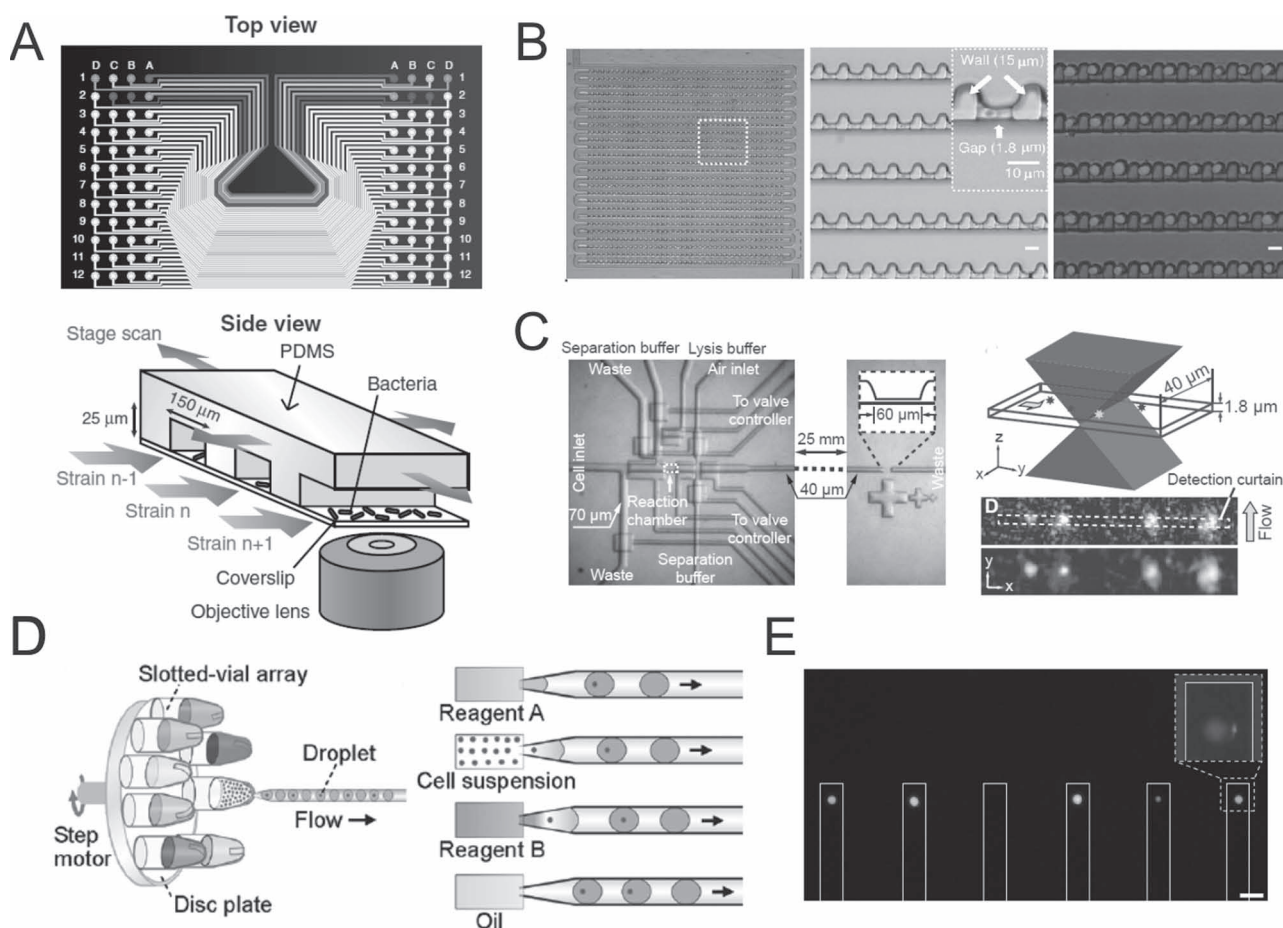


Figure 4. A. Microfluidic chip with multiple parallel channels for screening bacteria. Reproduced with permission.^[170] Copyright 2010, American Association for the Advancement of Science; B) Optical graphs and merged fluorescent images of single-cell trapping array. Reproduced with permission.^[183] Copyright 2011, American Chemical Society; C) Integrated valves for analyzing low-copy number proteins in single cells. Reproduced with permission.^[197] Copyright 2007, American Association for the Advancement of Science; D) Microfluidic droplet integrated with multi-step manipulation. Reproduced with permission.^[208] Copyright 2011, American Chemical Society; E) Non-adherent Jurkat cells captured by DNA barcode. Reproduced with permission.^[211] Copyright 2009, The Royal Society of Chemistry.

4.2.3. Droplet

The third method to trap single cells is droplet encapsulation.^[200–205] Droplet is an intriguing fluidic phenomenon, being discrete volumes with the use of immiscible in microchannels.^[206,207] Fang and Yao *et al.* developed a microfluidic droplet platform for implementing multistep solid extraction for analyzing single-cell enzyme activity and DNA purification (Figure 4D).^[208] Other works employed microfluidic droplet to trap and analyze subcellular organelles like mitochondria.^[209]

4.2.4. Surface Chemistry

In section 2.1.1, we introduced that surface chemistry could assist in cell patterning.^[54–56,58] When patterning size is down to single-cell level,^[57] surface chemistry could be a beneficial and efficient way to trap single cells. Mathies *et al.* developed two methods based on RGD peptide and ssDNA to capture single cells flowing over gold strip in microfluidic channels.^[175,210,211] ssDNA could capture non-adherent cells, such as primary T cell and Jurkat cells.^[211]

5. Long-Term Culture

Long-term culture enables prolonged (tens of days) observation and investigation of cellular processes on microfluidic chips.^[212] However, it confronts several challenges, two of which will be reviewed as follows.

One challenge for long-term culture is the constant supply of nutrients and removal of metabolic wastes. Levchenko *et al.* employed narrow microfluidic channels to restrict the movement of cells while allowing the diffusion of nutrients and soluble cues.^[213] This configuration realized a chemostat for growing cell colonies to high densities in expanded periods of time. Quake *et al.* and Takayama *et al.* employed movable components (including integrated PDMS valves and Braille displays) to ensure sustained nutrient supply.^[214–216] The former method also prevents the formation of biofilm and thus enables the observation of dynamics of cellular density. Considering the drawbacks of PDMS, Ren and Wu developed a whole Teflon chip that could provide both excellent anti-fouling surface and gas permeability for long-term cell culture.^[217] Most importantly, Teflon chip they manufactured

Table 2. Comparison of methods for single-cell trapping on microfluidic chips.

Target	Trapping method	Trapping efficiency	Throughput	Function/Suitability	Ref.
<i>E. coli</i> O157	Droplet	~20%	~95 cell/s	Gene analysis	[174]
<i>E. coli</i>	Integrated Valve	–	~160 cells/s	Proteome and Transcriptome	[170]
M1WT3 CHO cells	Surface Chemistry	63 ± 9% in 30 min 90 ± 5% in 60 min	–	Chemical stimuli	[210]
Pheochromocytoma cell (PC12)	Droplet	47%	–	DNA purification	[208]
Jurkat cells	Microarray	95%	800 cells per one trap	Chemical stimuli	[183]
T-cell lymphoma cell line (EG7)	Microarray	97%	–	Lineage tracking	[185]
U937 cells	Droplet	–	100 droplets/s	Screening drug	[201]

is elastic and transparent, representing huge potential to complement PDMS valves in many cases.

The other challenge to achieve long-term culture is the accumulation of air bubbles during the perfusion of medium into microfluidic chips, which would isolate cells in the air from liquid and eventually prevents from long-term culturing of cells. We reported a technology of integrated bubble trap (IBT) (Figure 2F).^[218] IBT is easy to fabricate, does not interfere with continuous fluid flow and allows MC 3T3 E1 cells to grow over 10 days under continuous flow.

6. Summary and Outlook

In summary, the energetic exploitation of inherent advantages of microfluidics results in the unprecedented achievements in manipulation and analysis of cells. There have been exciting achievements from analyzing low-copy number proteins in single cells, to high-throughput screening at one million cells per second and to constructing dynamic microenvironment. Such feat would significantly boost both fundamental biological studies and specific clinical applications. As microfluidics becomes increasingly useful for manipulating cells, there are some challenges that require much endeavor to clear the way for further development.

One challenge is the choice of materials to fabricate microfluidic chips. Most of works introduced in this review employed PDMS.^[219] Although PDMS is cytocompatible, elastic and offers rapid prototyping capabilities, it differs a lot from polystyrene (PS) that is commonly used in cell culture experiments. Some drawbacks of PDMS should be carefully considered when employing it for studying cells, such as the absorption of small hydrophobic molecules.^[220] Furthermore, the batch fabrication of PDMS-based microfluidic chips has not yet been realized.^[221] Novel techniques of chemical and physical modification to PDMS should be possible to make PDMS-based microfluidic chip more suitable for studying cells.^[222] Alternatively, engineering methods to integrate PS into microfluidic chips or just developing novel materials would be worth of more exploitations. The transparent and elastic Teflon microfluidic chip show definite promise in addressing this challenge.^[217]

Another challenge is the method to operate microfluidic chips. Currently, most of microfluidics chips are difficult

to operate for untrained personnel, and normally requires external equipments. To be adopted by common biology laboratories, some of microfluidic chips should be as easy as multi-well plate or centrifuge to operate.

Regardless of these challenges, the future of microfluidics seems rosy and promising. Microfluidic chips for cell screening possess notable merits in miniaturization and automation, and thus could underpin the development of personalized medical devices. Notably, microfluidic chips made in hydrogel could serve as novel tissue scaffolds with capabilities to mimic vasculature, which is a long-standing issue in tissue engineering and regenerative medicine.^[223,224]

At last, we would like to emphasize that the deep cooperation between biologists, chemists, engineers and clinicians is the key for utilizing microfluidics to solve biological/medical problems in real world.

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- [1] G. M. Whitesides, *Nature* **2006**, 442, 368.
- [2] S. C. Terry, J. H. Jerman, J. B. Angell, *IEEE Trans. Electron Devices* **1979**, 26, 1880.
- [3] A. Manz, Y. Miyahara, J. Miura, Y. Watanabe, H. Miyagi, K. Sato, *Sens. Actuator B* **1990**, 1, 249.
- [4] D. J. Harrison, K. Fluri, K. Seiler, Z. H. Fan, C. S. Effenhauser, A. Manz, *Science* **1993**, 261, 895.
- [5] J. El-Ali, P. K. Sorger, K. F. Jensen, *Nature* **2006**, 442, 403.
- [6] I. Meyvantsson, D. J. Beebe, *Annu. Rev. Anal. Chem.* **2008**, 1, 423.
- [7] A. Khademhosseini, R. Langer, J. Borenstein, J. P. Vacanti, *Proc. Natl. Acad. Sci. USA* **2006**, 103, 2480.
- [8] D. B. Weibel, W. R. DiLuzio, G. M. Whitesides, *Nat. Rev. Microbiol.* **2007**, 5, 209.

- [9] S. M. Kim, S. H. Lee, K. Y. Suh, *Lab Chip* **2008**, *8*, 1015.
- [10] E. W. K. Young, D. J. Beebe, *Chem. Soc. Rev.* **2010**, *39*, 1036.
- [11] L. Kim, Y. C. Toh, J. Voldman, H. Yu, *Lab Chip* **2007**, *7*, 681.
- [12] M. L. Kovarik, P. C. Gach, D. M. Orloff, Y. L. Wang, J. Balowski, L. Farrag, N. L. Allbritton, *Anal. Chem.* **2012**, *84*, 516.
- [13] C. E. Sims, N. L. Allbritton, *Lab Chip* **2007**, *7*, 423.
- [14] C. S. Chen, M. Mrksich, S. Huang, G. M. Whitesides, D. E. Ingber, *Science* **1997**, *276*, 1425.
- [15] K. A. Kilian, B. Bugarija, B. T. Lahn, M. Mrksich, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 4872.
- [16] H. Onoe, S. C. Hsiao, E. S. Douglas, Z. J. Gartner, C. R. Bertozzi, M. B. Francis, R. A. Mathies, *Langmuir* **2012**, *28*, 8120.
- [17] J. Park, D. H. Kim, G. Kim, Y. Kim, E. Choi, A. Levchenko, *Lab Chip* **2010**, *10*, 2130.
- [18] A. Khademhosseini, G. Eng, J. Yeh, P. A. Kucharczyk, R. Langer, G. Vunjak-Novakovic, M. Radisic, *Biomed. Microdevices* **2007**, *9*, 149.
- [19] X. Y. Jiang, D. A. Bruzewicz, A. P. Wong, M. Piel, G. M. Whitesides, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 975.
- [20] Y. Sun, Y. Y. Liu, W. S. Qu, X. Y. Jiang, *Anal. Chim. Acta* **2009**, *650*, 98.
- [21] W. W. Liu, Z. L. Chen, X. Y. Jiang, *Chin. J. Anal. Chem.* **2009**, *37*, 943.
- [22] Y. Sun, Z. Huang, K. X. Yang, W. W. Liu, Y. Y. Xie, B. Yuan, W. Zhang, X. Y. Jiang, *PLOS One* **2011**, *6*, e28156.
- [23] J. Y. Wang, L. Ren, L. Li, W. M. Liu, J. Zhou, W. H. Yu, D. W. Tong, S. L. Chen, *Lab Chip* **2009**, *9*, 644.
- [24] S. G. Xing, W. W. Liu, Z. Huang, L. Chen, K. Sun, D. Han, W. Zhang, X. Y. Jiang, *Electrophoresis* **2010**, *31*, 3144.
- [25] Y. Sun, Z. Huang, W. Liu, K. Yang, K. Sun, S. Xing, D. Wang, W. Zhang, X. Jiang, *Biointerphases* **2012**, *7*, 1.
- [26] S. R. Khetani, S. N. Bhatia, *Nat. Biotechnol.* **2008**, *26*, 120.
- [27] E. Ghafar-Zadeh, J. R. Waldeisen, L. P. Lee, *Lab Chip* **2011**, *11*, 3031.
- [28] W. M. Liu, L. Li, X. M. Wang, L. Ren, X. Q. Wang, J. C. Wang, Q. Tu, X. W. Huang, J. Y. Wang, *Lab Chip* **2010**, *10*, 1717.
- [29] W. M. Liu, P. Sun, L. Y. Yang, J. F. Wang, L. Li, J. Y. Wang, *Microfluid. Nanofluid.* **2010**, *9*, 717.
- [30] M. Gan, Y. Tang, Y. Shu, H. Wu, L. Chen, *Small* **2012**, *8*, 863.
- [31] T. M. Squires, S. R. Quake, *Rev. Mod. Phys.* **2005**, *77*, 977.
- [32] X. Mu, Q. L. Liang, P. Hu, K. N. Ren, Y. M. Wang, G. A. Luo, *Lab Chip* **2009**, *9*, 1994.
- [33] X. Mu, Q. L. Liang, P. Hu, K. N. Ren, Y. M. Wang, G. A. Luo, *Microfluid. Nanofluid.* **2010**, *9*, 365.
- [34] S. Takayama, J. C. McDonald, E. Ostuni, M. N. Liang, P. J. A. Kenis, R. F. Ismagilov, G. M. Whitesides, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5545.
- [35] Y. S. Torisawa, B. Mosadegh, G. D. Luker, M. Morell, K. S. O'Shea, S. Takayama, *Integr. Biol.* **2009**, *1*, 649.
- [36] X. W. Huang, L. Li, Q. Tu, J. C. Wang, W. M. Liu, X. Q. Wang, L. Ren, J. Y. Wang, *Microfluid. Nanofluid.* **2011**, *10*, 1333.
- [37] S. K. W. Dertinger, X. Y. Jiang, Z. Y. Li, V. N. Murthy, G. M. Whitesides, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 12542.
- [38] H. Kaji, T. Yokoi, T. Kawashima, M. Nishizawa, *Lab Chip* **2010**, *10*, 2374.
- [39] S. Takayama, E. Ostuni, P. LeDuc, K. Naruse, D. E. Ingber, G. M. Whitesides, *Chem. Biol.* **2003**, *10*, 123.
- [40] S. Takayama, E. Ostuni, P. LeDuc, K. Naruse, D. E. Ingber, G. M. Whitesides, *Nature* **2001**, *411*, 1016.
- [41] Y. Kim, S. D. Joshi, W. C. Messner, P. R. LeDuc, L. A. Davidson, *PLOS One* **2011**, *6*, e18556.
- [42] M. Meier, E. M. Lucchetta, R. F. Ismagilov, *Lab Chip* **2010**, *10*, 2147.
- [43] S. Chung, R. Sudo, P. J. Mack, C. R. Wan, V. Vickerman, R. D. Kamm, *Lab Chip* **2009**, *9*, 269.
- [44] V. Vickerman, J. Blundo, S. Chung, R. Kamm, *Lab Chip* **2008**, *8*, 1468.
- [45] L. Li, Y. Nie, X. T. Shi, H. K. Wu, D. T. Ye, H. D. Chen, *Biomicrofluidics* **2011**, *5*.
- [46] E. M. Lucchetta, J. H. Lee, L. A. Fu, N. H. Patel, R. F. Ismagilov, *Nature* **2005**, *434*, 1134.
- [47] X. Jiang, G. Whitesides, *Eng. Life Sci.* **2003**, *3*, 475.
- [48] R. F. Ismagilov, A. D. Stroock, P. J. A. Kenis, G. Whitesides, H. A. Stone, *Appl. Phys. Lett.* **2000**, *76*, 2376.
- [49] D. T. Chiu, N. L. Jeon, S. Huang, R. S. Kane, C. J. Wargo, I. S. Choi, D. E. Ingber, G. M. Whitesides, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 2408.
- [50] K. Sun, L. S. Song, Y. Y. Xie, D. B. Liu, D. Wang, Z. Wang, W. S. Ma, J. S. Zhu, X. Y. Jiang, *Langmuir* **2011**, *27*, 5709.
- [51] Y. Liu, Y. Sun, H. Yan, X. Liu, W. Zhang, Z. Wang, X. Jiang, *Small* **2012**, *8*, 676.
- [52] M. Khabiry, B. G. Chung, M. J. Hancock, H. C. Soundararajan, Y. N. Du, D. Cropek, W. G. Lee, A. Khademhosseini, *Small* **2009**, *5*, 1186.
- [53] R. A. Chandra, E. S. Douglas, R. A. Mathies, C. R. Bertozzi, M. B. Francis, *Angew. Chem. Int. Ed.* **2006**, *45*, 896.
- [54] X. Y. Jiang, R. Ferrigno, M. Mrksich, G. M. Whitesides, *J. Am. Chem. Soc.* **2003**, *125*, 2366.
- [55] K. Sun, B. Jiang, X. Y. Jiang, *J. Electroanal. Chem.* **2011**, *656*, 223.
- [56] Y. Li, B. Yuan, H. Ji, D. Han, S. Q. Chen, F. Tian, X. Y. Jiang, *Angew. Chem. Int. Ed.* **2007**, *46*, 1094.
- [57] K. Jang, K. Sato, K. Mawatari, T. Konno, K. Ishihara, T. Kitamori, *Biomaterials* **2009**, *30*, 1413.
- [58] D. B. Liu, Y. Y. Xie, H. W. Shao, X. Y. Jiang, *Angew. Chem. Int. Ed.* **2009**, *48*, 4406.
- [59] Z. Chen, Y. Dai, Z. Dong, M. Li, X. Mu, R. Zhang, Z. Wang, W. Zhang, J. Lang, J. Leng, X. Jiang, *Integr. Biol.* **2012**, DOI: 10.1039/C2IB00172A.
- [60] B. Yuan, Y. Li, D. Wang, Y. Y. Xie, Y. Y. Liu, L. Cui, F. Q. Tu, H. Li, H. Ji, W. Zhang, X. Y. Jiang, *Adv. Funct. Mater.* **2010**, *20*, 3715.
- [61] Y. Y. Xie, W. Zhang, L. M. Wang, K. Sun, Y. Sun, X. Y. Jiang, *Lab Chip* **2011**, *11*, 2819.
- [62] A. Khademhosseini, J. Yeh, G. Eng, J. Karp, H. Kaji, J. Borenstein, O. C. Farokhzad, R. Langer, *Lab Chip* **2005**, *5*, 1380.
- [63] D. B. Liu, Y. Y. Xie, H. W. Shao, X. Y. Jiang, *Prog. Chem.* **2007**, *19*, 1965.
- [64] Z. L. Chen, Y. Li, W. W. Liu, D. Z. Zhang, Y. Y. Zhao, B. Yuan, X. Y. Jiang, *Angew. Chem. Int. Ed.* **2009**, *48*, 8303.
- [65] Z. L. Chen, W. Chen, B. Yuan, L. Xiao, D. B. Liu, Y. Jin, B. G. Quan, J. O. Wang, K. Ibrahim, Z. Wang, W. Zhang, X. Y. Jiang, *Langmuir* **2010**, *26*, 17790.
- [66] B. Yuan, Y. Jin, Y. Sun, D. Wang, J. S. Sun, Z. Wang, W. Zhang, X. Y. Jiang, *Adv. Mater.* **2012**, *24*, 890.
- [67] S. Chung, R. Sudo, I. K. Zervantonakis, T. Rimchala, R. D. Kamm, *Adv. Mater.* **2009**, *21*, 4863.
- [68] C. P. Huang, J. Lu, H. Seon, A. P. Lee, L. A. Flanagan, H. Y. Kim, A. J. Putnam, N. L. Jeon, *Lab Chip* **2009**, *9*, 1740.
- [69] T. J. Liu, B. C. Lin, J. H. Qin, *Lab Chip* **2010**, *10*, 1671.
- [70] T. J. Liu, C. Y. Li, H. J. Li, S. J. Zeng, J. H. Qin, B. C. Lin, *Electrophoresis* **2009**, *30*, 4285.
- [71] D. A. Bruzewicz, A. P. McGuigan, G. M. Whitesides, *Lab Chip* **2008**, *8*, 663.
- [72] K. H. Lee, S. J. Shin, Y. Park, S. H. Lee, *Small* **2009**, *5*, 1264.
- [73] N. W. Choi, M. Cabodi, B. Held, J. P. Gleghorn, L. J. Bonassar, A. D. Stroock, *Nat. Mater.* **2007**, *6*, 908.
- [74] Y. Ling, J. Rubin, Y. Deng, C. Huang, U. Demirci, J. M. Karp, A. Khademhosseini, *Lab Chip* **2007**, *7*, 756.
- [75] B. Mosadegh, M. Agarwal, H. Tavana, T. Bersano-Begey, Y. Torisawa, M. Morell, M. J. Wyatt, K. S. O'Shea, K. F. Barald, S. Takayama, *Lab Chip* **2010**, *10*, 2959.
- [76] A. Shamloo, S. C. Heilshorn, *Lab Chip* **2010**, *10*, 3061.
- [77] J. M. Yang, J. E. Didier, T. R. Cassino, P. R. LeDuc, *Small* **2009**, *5*, 1984.

- [78] X. Y. Jiang, J. M. K. Ng, A. D. Stroock, S. K. W. Dertinger, G. M. Whitesides, *J. Am. Chem. Soc.* **2003**, *125*, 5294.
- [79] X. Y. Jiang, Q. B. Xu, S. K. W. Dertinger, A. D. Stroock, T. M. Fu, G. M. Whitesides, *Anal. Chem.* **2005**, *77*, 2338.
- [80] K. Sun, Z. X. Wang, X. Y. Jiang, *Lab Chip* **2008**, *8*, 1536.
- [81] H. K. Wu, B. Huang, R. N. Zare, *J. Am. Chem. Soc.* **2006**, *128*, 4194.
- [82] M. R. Bennett, W. L. Pang, N. A. Ostroff, B. L. Baumgartner, S. Nayak, L. S. Tsimring, J. Hasty, *Nature* **2008**, *454*, 1119.
- [83] J. Stricker, S. Cookson, M. R. Bennett, W. H. Mather, L. S. Tsimring, J. Hasty, *Nature* **2008**, *456*, 516.
- [84] O. Mondragon-Palomino, T. Danino, J. Selimkhanov, L. Tsimring, J. Hasty, *Science* **2011**, *333*, 1315.
- [85] N. Wang, J. P. Butler, D. E. Ingber, *Science* **1993**, *260*, 1124.
- [86] A. J. Engler, S. Sen, H. L. Sweeney, D. E. Discher, *Cell* **2006**, *126*, 677.
- [87] C. Mohrdieck, F. Dalmás, E. Arzt, R. Tharmann, M. Claessens, A. R. Bausch, A. Roth, E. Sackmann, C. H. J. Schmitz, J. Curtis, W. Roos, S. Schulz, K. Uhrig, J. P. Spatz, *Small* **2007**, *3*, 1015.
- [88] W. C. Ruder, E. D. Pratt, S. Bakhru, M. Sitti, S. Zappe, C.-M. Cheng, J. F. Antaki, P. R. LeDuc, *Lab Chip* **2012**, *12*, 1775.
- [89] C. Moraes, J. H. Chen, Y. Sun, C. A. Simmons, *Lab Chip* **2010**, *10*, 227.
- [90] D. Wang, Y. Y. Xie, B. Yuan, J. Xu, P. Y. Gong, X. Y. Jiang, *Integr. Biol.* **2010**, *2*, 288.
- [91] D. Huh, B. D. Matthews, A. Mammoto, M. Montoya-Zavala, H. Y. Hsin, D. E. Ingber, *Science* **2010**, *328*, 1662.
- [92] G. Perozziello, R. La Rocca, G. Cojoc, C. Liberale, N. Malara, G. Simone, P. Candeloro, A. Anichini, L. Tirinato, F. Gentile, M. L. Coluccio, E. Carbone, E. Di Fabrizio, *Small* **2012**, DOI: 10.1002/sml.201200160.
- [93] L. Chau, M. Doran, J. Cooper-White, *Lab Chip* **2009**, *9*, 1897.
- [94] W. Zheng, Y. Xie, W. Zhang, D. Wang, W. Ma, Z. Wang, X. Jiang, *Integr. Biol.* **2012**, DOI: 10.1039/C2IB20094E.
- [95] D. L. Butler, S. A. Goldstein, R. E. Guldborg, X. E. Guo, R. Kamm, C. T. Laurencin, L. V. McIntire, V. C. Mow, R. M. Nerem, R. L. Sah, L. J. Soslowsky, R. L. Spilker, R. T. Tranquillo, *Tissue Eng Part B* **2009**, *15*, 477.
- [96] W. Zheng, B. Jiang, D. Wang, W. Zhang, Z. Wang, X. Jiang, *Lab Chip* **2012**, DOI: 10.1039/C2LC40173H.
- [97] D. E. Discher, P. Janmey, Y. L. Wang, *Science* **2005**, *310*, 1139.
- [98] D. S. Gray, J. Tien, C. S. Chen, *J. Biomedical Mater. Res. Part A* **2003**, *66A*, 605.
- [99] S. Y. Chou, C. M. Cheng, C. C. Chen, P. R. LeDuc, *Soft Matter* **2011**, *7*, 9871.
- [100] P. G. Schiro, M. Zhao, J. S. Kuo, K. M. Koehler, D. E. Sabath, D. T. Chiu, *Angew. Chem. Int. Ed.* **2012**, *51*, 4618.
- [101] B. Yao, G. A. Luo, X. Feng, W. Wang, L. X. Chen, Y. M. Wang, *Lab Chip* **2004**, *4*, 603.
- [102] M. M. Wang, E. Tu, D. E. Raymond, J. M. Yang, H. C. Zhang, N. Hagen, B. Dees, E. M. Mercer, A. H. Forster, I. Kariv, P. J. Marchand, W. F. Butler, *Nat. Biotechnol.* **2005**, *23*, 83.
- [103] A. Salmazadeh, L. Romero, H. Shafiee, R. C. Gallo-Villanueva, M. A. Stremmer, S. D. Cramer, R. V. Davalos, *Lab Chip* **2012**, *12*, 182.
- [104] U. Kim, H. T. Soh, *Lab Chip* **2009**, *9*, 2313.
- [105] J. H. Kang, S. Krause, H. Tobin, A. Mammoto, M. Kanapathipillai, D. E. Ingber, *Lab Chip* **2012**, *12*, 2175.
- [106] J. D. Adams, U. Kim, H. T. Soh, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 18165.
- [107] N. Pamme, C. Wilhelm, *Lab Chip* **2006**, *6*, 974.
- [108] K. Hoshino, Y. Y. Huang, N. Lane, M. Huebschman, J. W. Uhr, E. P. Frenkel, X. J. Zhang, *Lab Chip* **2011**, *11*, 3449.
- [109] J. S. Kuo, Y. X. Zhao, P. G. Schiro, L. Y. Ng, D. S. W. Lim, J. P. Shelby, D. T. Chiu, *Lab Chip* **2010**, *10*, 837.
- [110] P. Sethu, A. Sin, M. Toner, *Lab Chip* **2006**, *6*, 83.
- [111] J. C. Baret, Y. Beck, I. Billas-Massobrio, D. Moras, A. D. Griffiths, *Chem. Biol.* **2010**, *17*, 528.
- [112] J. C. Baret, O. J. Miller, V. Taly, M. Ryckelynck, A. El-Harrak, L. Frenz, C. Rick, M. L. Samuels, J. B. Hutchison, J. J. Agresti, D. R. Link, D. A. Weitz, A. D. Griffiths, *Lab Chip* **2009**, *9*, 1850.
- [113] J. J. Agresti, E. Antipov, A. R. Abate, K. Ahn, A. C. Rowat, J. C. Baret, M. Marquez, A. M. Klibanov, A. D. Griffiths, D. A. Weitz, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 4004.
- [114] H. N. Joensson, M. Uhlen, H. A. Svahn, *Lab Chip* **2011**, *11*, 1305.
- [115] D. Di Carlo, *Lab Chip* **2009**, *9*, 3038.
- [116] N. Pamme, *Lab Chip* **2007**, *7*, 1644.
- [117] A. J. Mach, J. H. Kim, A. Arshi, S. C. Hur, D. Di Carlo, *Lab Chip* **2011**, *11*, 2827.
- [118] D. R. Gossett, D. Di Carlo, *Anal. Chem.* **2009**, *81*, 8459.
- [119] A. Jain, L. L. Munn, *Lab Chip* **2011**, *11*, 2941.
- [120] Z. G. Wu, B. Willing, J. Bjerketorp, J. K. Jansson, K. Hjort, *Lab Chip* **2009**, *9*, 1193.
- [121] D. Di Carlo, J. F. Edd, D. Irimia, R. G. Tompkins, M. Toner, *Anal. Chem.* **2008**, *80*, 2204.
- [122] A. A. S. Bhagat, H. W. Hou, L. D. Li, C. T. Lim, J. Y. Han, *Lab Chip* **2011**, *11*, 1870.
- [123] M. Chabert, J. L. Viovy, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 3191.
- [124] S. C. Hur, A. J. Mach, D. Di Carlo, *Biomicrofluidics* **2011**, *5*, 022206.
- [125] W. C. Lee, A. A. S. Bhagat, S. Huang, K. J. Van Vliet, J. Han, C. T. Lim, *Lab Chip* **2011**, *11*, 1359.
- [126] J. Warrick, B. Casavant, M. Frisk, D. Beebe, *Anal. Chem.* **2010**, *82*, 8320.
- [127] Z. Gu, Y. Tang, *Lab Chip* **2010**, *10*, 1946.
- [128] S. Nagrath, L. V. Sequist, S. Maheswaran, D. W. Bell, D. Irimia, L. Ulkus, M. R. Smith, E. L. Kwak, S. Digumarthy, A. Muzikansky, P. Ryan, U. J. Balis, R. G. Tompkins, D. A. Haber, M. Toner, *Nature* **2007**, *450*, 1235.
- [129] U. Dharmasiri, S. K. Njoroge, M. A. Witek, M. G. Adebisi, J. W. Kamande, M. L. Hupert, F. Barany, S. A. Soper, *Anal. Chem.* **2011**, *83*, 2301.
- [130] Y. Xu, J. A. Phillips, J. L. Yan, Q. G. Li, Z. H. Fan, W. H. Tan, *Anal. Chem.* **2009**, *81*, 7436.
- [131] L. Chen, X. L. Liu, B. Su, J. Li, L. Jiang, D. Han, S. T. Wang, *Adv. Mater.* **2011**, *23*, 4376.
- [132] W. Sheng, T. Chen, R. Kamath, X. Xiong, W. Tan, H. Fan, *Anal. Chem.* **2012**, *84*, 4199.
- [133] J. H. Myung, K. A. Gajjar, J. Saric, D. T. Eddington, S. Hong, *Angew. Chem. Int. Ed.* **2011**, *50*, 11769.
- [134] R. Schirhagl, E. W. Hall, I. Fuereder, R. N. Zare, *Analyst* **2012**, *137*, 1495.
- [135] K. Ren, R. N. Zare, *ACS Nano* **2012**, *6*, 4314.
- [136] R. Schirhagl, K. Ren, A. R. Zare, *Sci. China-Chem.* **2012**, *55*, 469.
- [137] G. D. Chen, F. Fachin, M. Fernandez-Suarez, B. L. Wardle, M. Toner, *Small* **2011**, *7*, 1061.
- [138] S. T. Wang, H. Wang, J. Jiao, K. J. Chen, G. E. Owens, K. I. Kamei, J. Sun, D. J. Sherman, C. P. Behrenbruch, H. Wu, H. R. Tseng, *Angew. Chem. Int. Ed.* **2009**, *48*, 8970.
- [139] S. T. Wang, K. Liu, J. A. Liu, Z. T. F. Yu, X. W. Xu, L. B. Zhao, T. Lee, E. K. Lee, J. Reiss, Y. K. Lee, L. W. K. Chung, J. T. Huang, M. Rettig, D. Seligson, K. N. Duraiswamy, C. K. F. Shen, H. R. Tseng, *Angew. Chem. Int. Ed.* **2011**, *50*, 3084.
- [140] S. L. Stott, C. H. Hsu, D. I. Tsukrov, M. Yu, D. T. Miyamoto, B. A. Waltman, S. M. Rothenberg, A. M. Shah, M. E. Smas, G. K. Korir, F. P. Floyd, A. J. Gilman, J. B. Lord, D. Winokur, S. Springer, D. Irimia, S. Nagrath, L. V. Sequist, R. J. Lee, K. J. Isselbacher, S. Maheswaran, D. A. Haber, M. Toner, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 18392.

- [141] S. T. Wang, K. Liu, J. Liu, Z. T. F. Yu, X. W. Xu, L. B. Zhao, T. Lee, E. K. Lee, J. Reiss, Y. K. Lee, L. W. K. Chung, J. T. Huang, M. Rettig, D. Seligson, K. N. Duraiswamy, C. K. F. Shen, H. R. Tseng, *Eur. Biophys. J. Biophys. Lett.* **2011**, *40*, 235.
- [142] J. Kaiser, *Science* **2010**, *327*, 1072.
- [143] M. Alunni-Fabroni, M. T. Sandri, *Methods* **2010**, *50*, 289.
- [144] M. N. Dickson, P. Tsinberg, Z. L. Tang, F. Z. Bischoff, T. Wilson, E. F. Leonard, *Biomicrofluidics* **2011**, *5*, 034119.
- [145] P. Li, Y. Gao, D. Pappas, *Anal. Chem.* **2011**, *83*, 7863.
- [146] D. J. Sherman, V. E. Kenanova, E. J. Lepin, K. E. McCabe, K. Kamei, M. Ohashi, S. T. Wang, H. R. Tseng, A. M. Wu, C. P. Behrenbruch, *Anal. Biochem.* **2010**, *401*, 173.
- [147] A. A. Adams, P. I. Okagbare, J. Feng, M. L. Hupert, D. Patterson, J. Gottert, R. L. McCarley, D. Nikitopoulos, M. C. Murphy, S. A. Soper, *J. Am. Chem. Soc.* **2008**, *130*, 8633.
- [148] S. Riethdorf, V. Muller, L. L. Zhang, T. Rau, S. Loibl, M. Komor, M. Roller, J. Huober, T. Fehm, I. Schrader, J. Hilfrich, F. Holms, H. Tesch, H. Eidtmann, M. Untch, G. von Minckwitz, K. Pantel, *Clin. Cancer Res.* **2010**, *16*, 2634.
- [149] M. Toner, D. Irimia, *Annu. Rev. Biomed. Eng.* **2005**, *7*, 77.
- [150] A. I. Rodriguez-Villarreal, M. Arundell, M. Carmona, J. Samitier, *Lab Chip* **2010**, *10*, 211.
- [151] I. K. Dimov, L. Basabe-Desmonts, J. L. Garcia-Cordero, B. M. Ross, A. J. Ricco, L. P. Lee, *Lab Chip* **2011**, *11*, 845.
- [152] J. P. Beech, S. H. Holm, K. Adolfsson, J. O. Tegenfeldt, *Lab Chip* **2012**, *12*, 1048.
- [153] S. Choi, S. Song, C. Choi, J. K. Park, *Small* **2008**, *4*, 634.
- [154] J. Nam, H. Lim, D. Kim, S. Shin, *Lab Chip* **2011**, *11*, 3361.
- [155] S. S. Shevkoplyas, T. Yoshida, L. L. Munn, M. W. Bitensky, *Anal. Chem.* **2005**, *77*, 933.
- [156] S. C. Hur, H. T. K. Tse, D. Di Carlo, *Lab Chip* **2010**, *10*, 274.
- [157] A. Y. Fu, H. P. Chou, C. Spence, F. H. Arnold, S. R. Quake, *Anal. Chem.* **2002**, *74*, 2451.
- [158] U. Kim, J. R. Qian, S. A. Kenrick, P. S. Daugherty, H. T. Soh, *Anal. Chem.* **2008**, *80*, 8656.
- [159] P. B. Lillehoj, H. Tsutsui, B. Valamehr, H. Wu, C. M. Ho, *Lab Chip* **2010**, *10*, 1678.
- [160] Z. G. Wu, K. Hjort, G. Wicher, A. F. Svenningsen, *Biomed. Microdevices* **2008**, *10*, 631.
- [161] S. S. Kuntaegowdanahalli, A. A. S. Bhagat, G. Kumar, I. Papautsky, *Lab Chip* **2009**, *9*, 2973.
- [162] C. W. Yung, J. Fiering, A. J. Mueller, D. E. Ingber, *Lab Chip* **2009**, *9*, 1171.
- [163] S. Reisewitz, H. Schroeder, N. Tort, K. A. Edwards, A. J. Baeumner, C. M. Niemeyer, *Small* **2010**, *6*, 2162.
- [164] E. Fitzpatrick, S. McBride, J. Yavelow, S. Najmi, P. Zanzucchi, R. Wieder, *Clin. Chem.* **2006**, *52*, 1080.
- [165] G. W. Li, X. S. Xie, *Nature* **2011**, *475*, 308.
- [166] D. L. Ryan, K. N. Ren, H. K. Wu, *Biomicrofluidics* **2011**, *5*, 021501.
- [167] R. N. Zare, S. Kim, Microfluidic in *Annual Review of Biomedical Engineering*, Vol 12 (Eds: M. L. Yarmush, J. S. Duncan, M. L. Gray), Annual Reviews: Palo Alto, **2010**, p.187.
- [168] C. Ma, R. Fan, H. Ahmad, Q. H. Shi, B. Comin-Anduix, T. Chodon, R. C. Koya, C. C. Liu, G. A. Kwong, C. G. Radu, A. Ribas, J. R. Heath, *Nat. Med.* **2011**, *17*, 738.
- [169] J. Sun, M. D. Masterman-Smith, N. A. Graham, J. Jiao, J. Mottahedeh, D. R. Laks, M. Ohashi, J. DeJesus, K. Kamei, K. B. Lee, H. Wang, Z. T. F. Yu, Y. T. Lu, S. A. Hou, K. Y. Li, M. Liu, N. G. Zhang, S. T. Wang, B. Angenieux, E. Panosyan, E. R. Samuels, J. Park, D. Williams, V. Konkankit, D. Nathanson, R. M. van Dam, M. E. Phelps, H. Wu, L. M. Liau, P. S. Mischel, J. A. Lazareff, H. I. Kornblum, W. H. Yong, T. G. Graeber, H. R. Tseng, *Cancer Res.* **2010**, *70*, 6128.
- [170] Y. Taniguchi, P. J. Choi, G. W. Li, H. Y. Chen, M. Babu, J. Hearn, A. Emili, X. S. Xie, *Science* **2010**, *329*, 533.
- [171] K. I. Kamei, S. L. Guo, Z. T. F. Yu, H. Takahashi, E. Gschwend, C. Suh, X. P. Wang, J. G. Tang, J. McLaughlin, O. N. Witte, K. B. Lee, H. R. Tseng, *Lab Chip* **2009**, *9*, 555.
- [172] Z. T. F. Yu, K. I. Kamei, H. Takahashi, C. J. Shu, X. P. Wang, G. W. He, R. Silverman, C. G. Radu, O. N. Witte, K. B. Lee, H. R. Tseng, *Biomed. Microdevices* **2009**, *11*, 547.
- [173] K. I. Kamei, M. Ohashi, E. Gschwend, Q. Ho, J. Suh, J. H. Tang, Z. T. F. Yu, A. T. Clark, A. D. Pyle, M. A. Teitell, K. B. Lee, O. N. Witte, H. R. Tseng, *Lab Chip* **2010**, *10*, 1113.
- [174] Y. Zeng, R. Novak, J. Shuga, M. T. Smith, R. A. Mathies, *Anal. Chem.* **2010**, *82*, 3183.
- [175] R. Novak, Y. Zeng, J. Shuga, G. Venugopalan, D. A. Fletcher, M. T. Smith, R. A. Mathies, *Angew. Chem. Int. Ed.* **2011**, *50*, 390.
- [176] E. C. Jensen, Y. Zeng, J. Kim, R. A. Mathies, *Jala* **2010**, *15*, 455.
- [177] D. Di Carlo, L. Y. Wu, L. P. Lee, *Lab Chip* **2006**, *6*, 1445.
- [178] M. C. Kim, B. C. Isenberg, J. Sutin, A. Meller, J. Y. Wong, C. M. Klapperich, *Lab Chip* **2011**, *11*, 1089.
- [179] S. L. Faley, M. Copland, D. Wlodkowic, W. Kolch, K. T. Seale, J. P. Wikswo, J. M. Cooper, *Lab Chip* **2009**, *9*, 2659.
- [180] M. C. Park, J. Y. Hur, H. S. Cho, S. H. Park, K. Y. Suh, *Lab Chip* **2011**, *11*, 79.
- [181] M. Hosokawa, T. Hayashi, T. Mori, T. Yoshino, S. Nakasono, T. Matsunaga, *Anal. Chem.* **2011**, *83*, 3648.
- [182] J. F. Edd, D. Di Carlo, K. J. Humphry, S. Koster, D. Irimia, D. A. Weitz, M. Toner, *Lab Chip* **2008**, *8*, 1262.
- [183] K. H. Chung, C. A. Rivet, M. L. Kemp, H. Lu, *Anal. Chem.* **2011**, *83*, 7044.
- [184] S. H. Kim, T. Yamamoto, D. Fourmy, T. Fujii, *Small* **2011**, *7*, 3239.
- [185] S. Kobel, A. Valero, J. Latt, P. Renaud, M. Lutolf, *Lab Chip* **2010**, *10*, 857.
- [186] J. P. Frimat, M. Becker, Y. Y. Chiang, U. Marggraf, D. Janasek, J. G. Hengstler, J. Franzke, J. West, *Lab Chip* **2011**, *11*, 231.
- [187] S. Hong, Q. Pan, L. P. Lee, *Integr. Biol.* **2012**, *4*, 374.
- [188] D. Di Carlo, L. P. Lee, *Anal. Chem.* **2006**, *78*, 7918.
- [189] D. Di Carlo, N. Aghdam, L. P. Lee, *Anal. Chem.* **2006**, *78*, 4925.
- [190] A. R. Wheeler, W. R. Thronset, R. J. Whelan, A. M. Leach, R. N. Zare, Y. H. Liao, K. Farrell, I. D. Manger, A. Daridon, *Anal. Chem.* **2003**, *75*, 3581.
- [191] H. K. Wu, A. Wheeler, R. N. Zare, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12809.
- [192] Q. H. Shi, L. D. Qin, W. Wei, F. Geng, R. Fan, Y. S. Shin, D. L. Guo, L. Hood, P. S. Mischel, J. R. Heath, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 419.
- [193] J. Shen, Y. Zhou, T. Lu, J. Y. Peng, Z. X. Lin, L. Huang, Y. H. Pang, L. Yu, Y. Y. Huang, *Lab Chip* **2012**, *12*, 317.
- [194] L. F. Yu, H. Q. Huang, X. L. Dong, D. P. Wu, J. H. Qin, B. C. Lin, *Electrophoresis* **2008**, *29*, 5055.
- [195] C. X. Xu, X. F. Yin, *J. Chromatogr. A* **2011**, *1218*, 726.
- [196] J. S. Edgar, G. Milne, Y. Q. Zhao, C. P. Pabbati, D. S. W. Lim, D. T. Chiu, *Angew. Chem. Int. Ed.* **2009**, *48*, 2719.
- [197] B. Huang, H. K. Wu, D. Bhaya, A. Grossman, S. Granier, B. K. Kobilka, R. N. Zare, *Science* **2007**, *315*, 81.
- [198] W. Liu, L. Li, J.-c. Wang, Q. Tu, L. Ren, Y. Wang, J. Wang, *Lab Chip* **2012**, *12*, 1702.
- [199] L. Cai, N. Friedman, X. S. Xie, *Nature* **2006**, *440*, 358.
- [200] M. Y. He, J. S. Edgar, G. D. M. Jeffries, R. M. Lorenz, J. P. Shelby, D. T. Chiu, *Anal. Chem.* **2005**, *77*, 1539.
- [201] E. Brouzes, M. Medkova, N. Savenelli, D. Marran, M. Twardowski, J. B. Hutchison, J. M. Rothberg, D. R. Link, N. Perimon, M. L. Samuels, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 14195.
- [202] S. Koster, F. E. Angile, H. Duan, J. J. Agresti, A. Wintner, C. Schmitz, A. C. Rowat, C. A. Merten, D. Pisignano, A. D. Griffiths, D. A. Weitz, *Lab Chip* **2008**, *8*, 1110.
- [203] J. Clausell-Tormos, D. Lieber, J. C. Baret, A. El-Harrak, O. J. Miller, L. Frenz, J. Blouwolff, K. J. Humphry, S. Koster, H. Duan, C. Holtze, D. A. Weitz, A. D. Griffiths, C. A. Merten, *Chem. Biol.* **2008**, *15*, 427.

- [204] W. H. Tan, S. Takeuchi, *Adv. Mater.* **2007**, *19*, 2696.
- [205] D. T. Chiu, *Anal. Bioanal. Chem.* **2010**, *397*, 3179.
- [206] S. Y. Teh, R. Lin, L. H. Hung, A. P. Lee, *Lab Chip* **2008**, *8*, 198.
- [207] M. Joanicot, A. Ajdari, *Science* **2005**, *309*, 887.
- [208] S. Q. Gu, Y. X. Zhang, Y. Zhu, W. B. Du, B. Yao, Q. Fang, *Anal. Chem.* **2011**, *83*, 7570.
- [209] J. C. Gadd, C. L. Kuyper, B. S. Fujimoto, R. W. Allen, D. T. Chiu, *Anal. Chem.* **2008**, *80*, 3450.
- [210] N. M. Toriello, E. S. Douglas, R. A. Mathies, *Anal. Chem.* **2005**, *77*, 6935.
- [211] E. S. Douglas, S. C. Hsiao, H. Onoe, C. R. Bertozzi, M. B. Francis, R. A. Mathies, *Lab Chip* **2009**, *9*, 2010.
- [212] J. H. Zhou, K. N. Ren, W. Dai, Y. H. Zhao, D. Ryan, H. K. Wu, *Lab Chip* **2011**, *11*, 2288.
- [213] A. Groisman, C. Lobo, H. J. Cho, J. K. Campbell, Y. S. Dufour, A. M. Stevens, A. Levchenko, *Nat. Methods* **2005**, *2*, 685.
- [214] W. Gu, X. Y. Zhu, N. Futai, B. S. Cho, S. Takayama, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15861.
- [215] F. K. Balagadde, L. C. You, C. L. Hansen, F. H. Arnold, S. R. Quake, *Science* **2005**, *309*, 137.
- [216] S.-J. Kim, D. Lai, J. Y. Park, R. Yokokawa, S. Takayama, *Small* **2012**, DOI: 10.1002/smll.201200456.
- [217] K. N. Ren, W. Dai, J. H. Zhou, J. Su, H. K. Wu, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 8162.
- [218] W. F. Zheng, Z. Wang, W. Zhang, X. Y. Jiang, *Lab Chip* **2010**, *10*, 2906.
- [219] S. K. Sia, G. M. Whitesides, *Electrophoresis* **2003**, *24*, 3563.
- [220] E. Berthier, E. W. K. Young, D. Beebe, *Lab Chip* **2012**, *12*, 1224.
- [221] R. Mukhopadhyay, *Anal. Chem.* **2007**, *79*, 3248.
- [222] J. W. Zhou, D. A. Khodakov, A. V. Ellis, N. H. Voelcker, *Electrophoresis* **2012**, *33*, 89.
- [223] M. Lovett, K. Lee, A. Edwards, D. L. Kaplan, *Tissue Eng. Part B* **2009**, *15*, 353.
- [224] A. Khademhosseini, R. Langer, *Biomaterials* **2007**, *28*, 5087.

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